

EDITORIAL COMMENT

Low Levels of High-Density Lipoprotein Cholesterol Due to Lecithin:Cholesterol Acyltransferase Mutations Increase Carotid Atherosclerosis*

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Despite the long-held knowledge of an inverse relationship between plasma high-density lipoprotein (HDL) cholesterol levels and rates of cardiovascular disease (CVD) in populations, the HDL hypothesis has recently been shaken to its foundations by a barrage of controversial data. For example, clinical trials of an agent (torcetrapib) that increases HDL cholesterol by inhibiting cholesteryl ester transfer protein (CETP), an essential transferase that moves cholesterol from HDL to apolipoprotein B (apo B)-containing lipoproteins, have shown unexpected harm with increased cardiovascular events after <1 year of treatment among subjects who were already taking protective doses of atorvastatin (1). In addition, coronary and carotid imaging studies with the same agent failed to show vascular improvements attributable to the drug or to the increase in HDL cholesterol (2,3). Moreover, genetic studies have challenged

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the role of HDL in atherogenesis by providing observations contrary to the dogma that HDL is a protector of the artery wall. For example, mutations in CETP or hepatic lipase (HL), both causing significant increases in HDL cholesterol presumably sustained over the lifetime of the carriers, are associated with increased cardiovascular event rates compared with non-carriers (4–6). It is thus particularly important that the study by Duivenvoorden et al. (7) reported in this issue of the *Journal* provides support for the previously intuitive concept that low HDL increases the risk of CVD. In this study, 40 subjects (38 heterozygotes, 2 homozygotes) with mutations in the gene for

lecithin:cholesterol acyltransferase (LCAT) were compared with 40 controls (both family members and unrelated individuals) matched for age and cardiovascular risk factors. Subjects and controls were, on average, 42 years old, borderline overweight, nonsmokers, and moderate alcohol users. One-half of the carriers were taking lipid-lowering drugs (mostly statins) compared with only 1 user among the controls. The lipid panel showed no differences between groups in total cholesterol, low-density lipoprotein (LDL) cholesterol, and apo B levels. As expected, carriers had much lower HDL cholesterol and apolipoprotein A-I (apo A-I) levels compared with controls (34 mg/dl vs. 54 mg/dl and 119 mg/dl vs. 150 mg/dl, respectively), attributable to a 28% reduction in plasma LCAT activity. The absence of differences in LDL cholesterol levels goes along with the phenotype of incomplete LCAT deficiency, in which only the transferase activity linked to HDL is defective (fish eye disease). In cases of complete LCAT deficiency (familial LCAT deficiency), the transferase activity associated with LDL is also lost, and, as a consequence, LDL levels are reduced (8).

In this study, the outcome measure was carotid wall thickness, and the evaluation was based on 2 imaging modalities: 3.0-T carotid magnetic resonance imaging (MRI) and classic B-mode ultrasound. Both MRI and B-mode ultrasound data imaging were analyzed by blinded operators. The results are compatible with a negative effect of the genetically determined low HDL on carotid artery wall thickening. The carriers had a 10% higher normalized wall index (primary outcome) and a 20% higher mean wall area and total wall volume (secondary outcomes). In addition, exploratory endpoints for the MRI analyses showed that 50% of the carriers and 8% of controls had atherosclerotic plaque components defined as either lipid-rich tissue or calcified material. There was also a significant difference in total plaque volume (102 mm³ vs. 3 mm³ in carriers vs. noncarriers, respectively). All these differences were significant after adjustment for the major risk factors including age, sex, blood pressure, body mass index, LDL cholesterol, smoking, and family history of CVD. Interestingly, the measurement with B-mode US (for common carotid intima-media thickness [CCIMT]) showed a trend for an increase in carriers versus controls of 12.5% (0.72 mm vs. 0.64 mm), but this difference did not come close to statistical significance ($p = 0.14$) even though it was similar in size to the difference between groups in the MRI study.

The knowledge that genetically determined low HDL cholesterol levels are associated with increased development of carotid plaques provides a ray of light for investigators and enterprises hard at work identifying a valid therapeutic target to increase plasma HDL cholesterol or, better yet, to improve reverse cholesterol transport (RCT). However, these newest data are far from being unchallenged and do not definitely put the case to rest. Similar studies have been done in the past with carriers of dysfunctional LCAT mutants or by evaluating LCAT activity as a predictor of CVD in populations or as associated parameters in patients with CVD. Two previous

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studies of B-mode ultrasound investigation of carotid disease among carriers of LCAT mutations provided opposite results. One study, from the laboratory that also conducted the current study, found an increased CCIMT among Dutch carriers of LCAT mutations causing fish eye disease compared with controls (9). Another study by Calabresi et al. (10) found that Italian carriers of LCAT mutations causing familial LCAT deficiency had a significantly decreased CCIMT. This puts into question 2 issues: 1) the value of other lipid parameters in determining the atherogenicity of HDL reduction due to LCAT mutations and 2) the sensitivity of B-mode ultrasound. In fact, in the study by Calabresi et al. (10), the carriers of LCAT mutations had lower LDL levels than controls because of the absence of functional LCAT on apo B-containing lipoproteins. This suggests that sustained low LDL cholesterol levels prevail over sustained low HDL cholesterol levels in predicting vascular changes, even in subjects whose exclusive lipid abnormality is low HDL cholesterol. This would certainly be in line with the current view of LDL as primary target of lipid therapy for CVD risk reduction. It is also possible that the divergent results of the 2 studies are explained by inherent technical limitations of B-mode ultrasound. This possibility is supported by the fact that in the current study, B-mode ultrasound was not able to demonstrate a significant effect of LCAT mutations and lifetime low HDL on CCIMT. Had this been another ultrasound-only study, it would have only added to the confusion. This is a strong reminder that more advanced imaging approaches are necessary to delineate the role of single gene contributors to a complex disease such as pre-clinical atherosclerosis.

Population and clinical studies, by and large, have eliminated a major role for variations in LCAT activity or mass as predictors of CVD (11,12). In the experimental world, results have been equally uninformative and confusing. Deletion of LCAT in mice has been linked to both increased and decreased atherosclerosis, depending on the levels of apo B-containing lipoproteins (13). Overexpression of LCAT in transgenic mice produced an expected increase in HDL cholesterol and an unexpected impairment in RCT (41% reduction in the return of HDL cholesterol to the liver) with paradoxically increased atherosclerotic plaque volume (14). It has to be noted that the mouse does not have CETP activity in plasma and that its HL is mostly circulating rather than liver bound. When LCAT was overexpressed in rabbits, which, like humans, express CETP and have liver-bound HL, the increased HDL cholesterol was linked with significant reduction in atherosclerotic burden. Of note, when CETP was coexpressed in LCAT transgenic mice, the negative vascular effects were deleted, and the phenotype reverted to show reduction in atherosclerosis (15). These data make it clear that therapeutic exploitation of the RCT must be carefully orchestrated in consideration of diverging results that may be collected depending on the status of different components of the system. For example, activation of LCAT under conditions of inhibited CETP (with niacin therapy at present, CETP inhibitors maybe in the future) (16,17) may produce altered plasma cholesterol flux with

ultimately negative effects on vascular health. This notwithstanding, it is encouraging to see an important regulator of RCT confirming the dangers of low HDL cholesterol levels and consolidating its position as a valid therapeutic target. In this regard, it is worth noting that 2 groups have recently reported work on the use of recombinant LCAT in experimental animals. One group has studied the effect of injecting recombinant LCAT in primates and showed significant improvement in plasma LDL and HDL levels (18). Closer to the therapeutic level, another group has recently reported the production of a hyperfunctional engineered LCAT (about 7-fold more active than normal human LCAT) that increased HDL cholesterol 3-fold and reduced cholesterol accumulation in aortic plaques after infusion in rabbits (19).

The contribution of HDL to RCT can be separated into 2 stages: 1) the stage of cholesterol acquisition from peripheral tissues, which requires the presence of abundant amounts of the cholesterol acceptor apo A-I in the form of pre-beta HDL particles, the up-regulation of cellular lipid transporters such as ABCA1 and ABCG1 to facilitate export of intracellular cholesterol to HDL, and the activity of LCAT, which efficiently esterifies cellular free cholesterol to promote particle maturation via expansion of the HDL core; and 2) the stage of cholesterol delivery, which involves CETP action to transfer cholesterol to apo B-containing lipoproteins in exchange for triglycerides, remodeling of the HDL particle by action of HL, and interaction between apo A-I and the HDL receptor SR-BI to deliver HDL's cholesterol cargo to liver cells.

Therapeutic HDL cholesterol increases can be accomplished by facilitating one of the steps of stage 1 or by inhibiting one of the steps of stage 2. Despite the negative results seen with the first CETP inhibitor, active investigation is in place with newer molecules, 2 of which apparently lack toxic off-target effects (20,21). Some emphasis is also being placed on inhibitors of SR-BI, even though mouse data suggest that the increase in HDL caused by the loss of SR-BI may not be protective (22). A safer avenue of intervention, at least on theoretical grounds, is offered by interventions improving the efficiency of the steps involved in stage 1, aiding cholesterol exit from peripheral cells, and HDL formation and maturation. Multiple approaches are being used to achieve this goal, including small molecules activating apo A-I production, infusion of small recombinant HDL, and reinfusion of delipidated HDL (pre-beta particles) (23). The study by Duivenvoorden et al. (7) provides much needed support for the idea that activation of LCAT may be exploited as a way to increase HDL cholesterol levels, improve RCT, and enhance vascular homeostasis.

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